ATENT COOPERATION TRE..TY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/025477	06.08.2004	06.08.2003

International Patent Classification (IPC) or both national classification and IPC
A61K39/02, A61K39/385, A61K39/39

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA....

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Perez, F

Telephone No. +49 89 2399-7338

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/025477

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18, 27-30, 32
	No:	Claims	19-26, 31
Inventive step (IS)	Yes:	Claims	13-18, 27-30
	No:	Claims	1-12, 19-26, 31-32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations

   see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No. PCT/US2004/025477

Re Item IV

Lack of unity of invention

This international Searching Authority found multiple (group of) inventions in this international application as indicated below:

1 Claims 1-8, 19-22 (all completely), 31-32 (in part) directed to conjugate vaccines and method for preparing them by reacting an aldehyde activated polysaccharide (PS) with a hydrazine activated protein.

1.1 Claims 9-12, 23-26 (all completely), 31-32 (in part) directed to conjugate vaccines and method for preparing them by reacting a CDAP activated polysaccharide with a hydrazine activated protein.

2 Claims 13-18, 27-30 (all completely), 31-32 (in part) directed to conjugate vaccines and method for preparing them by reacting a hydrazide activated polysaccharide with a APDO activated protein.

The reason for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT are as follow:

The general concept underlying inventions 1, 1.1 and 2 is the use of hydrazide chemistry in the chemical bridging of a polysaccharide with a protein to prepare a conjugate vaccine.

However, this concept is known from the prior art, and is disclosed for instance in the following documents:

Shafer et al, Vaccine (18) 2000, 1273-1281 discloses CDAP activated polysaccharides conjugated to hydrazine activated protein (see § 3.1 to 3.5)

Konadu et al, Infection and Immunity, 2000, Vol 18, No. 3, p1529-1534 discloses CDAP activated polysaccharides conjugated to protein via an adipic acid hydrazide linker (see page 1529, right column)

The technical problem underlying the application may thus be defined as providing further conjugate vaccine and methods to prepare them.

In view of the absence of any additional common feature which could be seen as a "special technical feature" in the sense of Rule 13.2 PCT, each invention 1, 1.1 and 2 represent a different solution to the given technical problem.

The requirements for unity of invention are therefore not fulfilled.

Please note that inventions mentioned under item 1 and 1.1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee. In response to the invitation, the applicant has paid one additional search fee for invention 2.

A search report has been established for inventions 1 and 1.1 and 2. An opinion will thus be given for subject matter corresponding to claims 1-32.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: LEE CHI-JEN: "Quality control of polyvalent pneumococcal polysaccharide-protein conjugate vaccine by nephelometry" BIOLOGICALS, vol. 30, no. 2, June 2002 (2002-06), pages 97-103

D2: US-A-4 356 170 (JENNINGS ET AL) 26 October 1982 (1982-10-26)

D3: SHAFER DOUGLAS E ET AL: "Activation of soluble polysaccharides with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) for use in protein-polysaccharide conjugate vaccines and immunological reagents. II. Selective crosslinking of proteins to CDAP-activated polysaccharides" VACCINE, vol. 18, no. 13, January 2000 (2000-01), pages 1273-1281

D4: LEES A ET AL: "Activation of soluble polysaccharides with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate for use in protein-polysaccharide conjugate vaccines and immunological reagents." VACCINE. FEB 1996, vol. 14, no. 3, February 1996 (1996-02), pages 190-198

D5: KONADU EDWARD Y ET AL: "Phase 1 and phase 2 studies of Salmonella enterica serovar Paratyphi A O-specific polysaccharide-tetanus toxoid conjugates in adults, teenagers, and 2- to 4-year-old children in Vietnam" INFECTION AND IMMUNITY, vol. 68, no. 3, March 2000 (2000-03), pages 1529-1534

D6: MULARD L ET AL: "Vaccins polyosidiques" ANNALES DE L'INSTITUT PASTEUR ACTUALITES, vol. 12, no. 2, May 2002 (2002-05), pages 37-54

Novelty and inventive step for invention 1 (Articles 33.1, 33.2 and 33.2 PCT)

Claims 1-8, 19-22, 31-32 (in part) are directed to conjugate vaccines and method for preparing them by reacting an aldehyde activated polysaccharide (CHO-PS) with a hydrazine activated protein.

Document D1 and D2 disclose similar methods. Nevertheless, the following differences can be found between the methods described in the prior art and the method disclosed in the present application:

D1 does not disclose the following steps:

(a) buffer exchanging the CHO-PS at pH 7-8,
(b) raising the pH of the hydrazine activated protein to pH 7-11,
(c) buffer exchanging the hydrazine activated protein at pH 10-11.

D2 does not disclose the following steps:

(a) buffer exchanging the CHO-PS at pH 7-8,
(b) raising the pH of the hydrazine activated protein to pH 7-11,
(c) buffer exchanging the hydrazine activated protein at pH 10-11.

In addition D2 differs from the present application in the following step

(d) reacting the CHO-PS with the hydrazine activated protein at pH 6-8.

The subject matter of claims 1-8 is therefore new over the cited prior art.

The compounds obtained by the methods disclosed in D1 and D2 are identical to those claimed in claims 19-22. Claims 19-22 therefore lack novelty.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-8. does not involve an inventive step in the sense of Article 33(3) PCT.

The document D1 is regarded as being the closest prior art to the subject-matter of claim 1-8, and discloses method to prepare conjugate vaccines from which the subject-matter of claim 1-8 differs in the aforementioned features (a), (b) and (c).

The problem to be solved by the present invention may therefore be regarded as providing a better conjugation method.

The solution proposed in claim 1-8 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

The aforementioned features (a), (b) and (c) would be regarded by the skilled person as a normal option to include in the method disclosed by D1.

In addition, the aforementioned features (a), (b) and (c) cannot be linked to any surprising and advantageous effect.

The subject matter of claims 1-8 therefore lacks an inventive step.

Novelty and inventive step for invention 1.1 (Articles 33.1, 33.2 and 33.2 PCT)

Claims 9-12, 23-26 and 31-32 (in part) are directed to conjugate vaccines and method for preparing them by reacting 1-cyano-4-dimethylammoniumpyridinium tetrafluoroborate activated polysaccharide (CDAP-PS) with a hydrazine activated protein.

Document D3, D4 and D5 disclose similar methods. Nevertheless, the following differences can be found between the methods described in the prior art and the method disclosed in the

present application:

D3 does not disclose the following steps

(e) reacting a protein with hydrazine or adipic acid dihydrazine (ADH) at pH 6-7

(f) raising the pH of the hydrazine activated protein to pH 7-11,

(g) buffer exchanging the hydrazine activated protein at pH 10-11.

D4 does not disclose the following steps

(e) reacting a protein with hydrazine or adipic acid dihydrazine (ADH) at pH 6-7

(f) raising the pH of the hydrazine activated protein to pH 7-11,

(g) buffer exchanging the hydrazine activated protein at pH 10-11.

D5 does not disclose the following steps

(e) reacting a protein with hydrazine or adipic acid dihydrazine (ADH) at pH 6-7

(f) raising the pH of the hydrazine activated protein to pH 7-11,

(g) buffer exchanging the hydrazine activated protein at pH 10-11.

In addition D5 differs from the present application in the following step

(h) reacting the CDAP-PS with the hydrazine activated protein at pH 6-8.

Claims 9-12 are therefore new over the cited prior art.

The compounds obtained by the methods disclosed in D3-D5 are identical to those claimed in claims 23-26. The subject matter of claims 23-26 therefore lacks novelty.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 9-12 does not involve an inventive step in the sense of Article 33(3) PCT.

The document D3 is regarded as being the closest prior art to the subject-matter of claim 9-12, and discloses method to prepare conjugate vaccines from which the subject-matter of claim 1-8 differs in the aforementioned features (e), (f) and (g).

The problem to be solved by the present invention may therefore be regarded as providing a better conjugation method.

The solution proposed in claim 9-12 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

The aforementioned features (e), (f) and (g) would be regarded by the skilled person as a normal option to include in the method disclosed by D3.

In addition, the aforementioned features (e), (f) and (g) cannot be linked to any surprising and advantageous effect.

The subject matter of claims 9-12 therefore lacks an inventive step.

Novelty and inventive step for invention 2 (Articles 33.1, 33.2 and 33.2 PCT)

Claims 13-18, 27-30 (all completely), 31-32 (in part) are directed to conjugate vaccines and method for preparing them by reacting a hydrazide activated polysaccharide with a APDO activated protein.

No document could be found describing APDO activation of a protein previous to conjugation with a polysaccharide. The subject matter of claims 13-18 is therefore new.

The compounds obtainable with the method of claims 13-18 are characterised by the presence of an hydrazine amide linker and are therefore different from the compounds obtainable by the processes of claims 1-8 and 9-12. No compound characterised by the linking group given in claim 27, nor conjugation methods succeptible to produce them, could be found in the prior art. The subject matter of claims 13-18, 27-30 is therefore novel.

The technical problem underlying the application, defined as providing further conjugate vaccine and methods to prepare them has been shown to be solved (see pages 43-44). No incentive of using APDO activation of the protein before the conjugation could be found in the prior art. Moreover, the conjugates obtainable with this method have the unexpected advantageous effect of inducing 886 fold antibodies as compared with a control conjugate vaccine. The subject matter of claims 13-18, 27-30 is therefore inventive.

Novelty and inventive step for claims 31-32 (Articles 33.1, 33.2 and 33.2 PCT)

Claim 31 refers to all conjugate vaccines as herein described. Claim 32 refers to all methods for preparing conjugate vaccines as herein described. Therefore, claims 32 is novel but lacks an inventive step, whereas claim 31 lacks novelty for the reasons detailed in the above chapters relating to inventions 1 and 1.1.

Industrial applicability (Articles 33.1 and 33.4 PCT)

Claims 1-32 relate to conjugate vaccines and methods to prepare them and are therefore susceptible of an industrial application.

Further remarks

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D6 is not mentioned in the description, nor are these documents identified therein.

The relative term "about" used in claims 1-32 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of the claims unclear, Article 6 PCT.

Claims 31-32 contain references to the description. According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.